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Bioorganic & Medicinal Chemistry Letters 17 (2007) 5525–5528
Bioorganic & Medicinal Chemistry Letters

Pyrazole-based cathepsin S inhibitors with improved cellular potency

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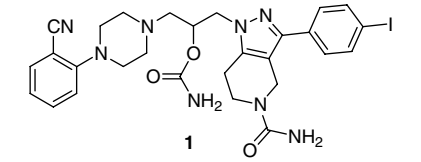
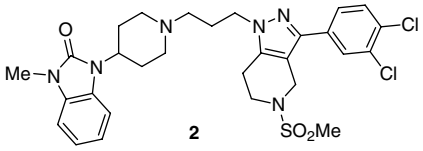
Received 3 July 2007; revised 13 August 2007; accepted 15 August 2007
Available online 22 August 2007

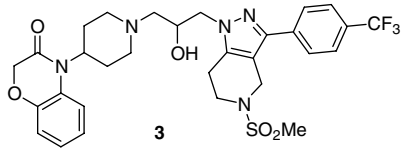
Abstract—High potency pyrazole-based noncovalent inhibitors of human cathepsin S (CatS) were developed by modification of the benzo-fused 5-membered ring heterocycles found in earlier series of CatS inhibitors. Although substitutions on this heterocyclic framework had a moderate impact on enzymatic potency, dramatic effects on cellular activity were observed. Optimization afforded indole- and benzothiophene-derived analogues that were high affinity CatS inhibitors (IC_{50} = 20–40 nM) with good cellular potency (IC_{50} = 30–340 nM).

Cathepsin S (CatS) is a cysteine protease of the papain family that is involved in the presentation of antigens to the cell surface of certain antigen-presenting cells (APCs) for recognition by $CD4^+$ T-cells. The main APC target of the proteolytic activity of CatS is the invariant chain (Ii), a chaperone molecule for major histocompatibility complex class II molecules (MHCII).[1–3] Inhibition of CatS activity impedes the removal of Ii from MHCII molecules, and thus attenuates antigen presentation to $CD4^+$ T-cells. Other pharmacologically relevant activities have also been attributed to CatS.[4] Numerous selective CatS inhibitor chemotypes have been reported, most relying upon covalent modification of the active site cysteine to achieve good activity.[5] Recently, potent noncovalent inhibitors have emerged from these laboratories[6] and from researchers at Novartis.[7,8]

We have previously reported high potency noncovalent inhibitors of human CatS based on a tetrahydropyrido-pyrazole heterocycle. Representative analogues with different aryl- and heteroaryl-substituted piperazine and piperidine groups are shown below.







While compounds **1**–**3** all inhibit human CatS with IC_{50}'s in the 10–20 nM range, they are much less active in a secondary cellular assay measuring Ii degradation in (human) JY cells (IC_{50} = 0.8–1.2 μM). We had earlier used to advantage the tolerance of CatS inhibitory potency to structural variability in the aryl/heteroaryl groups found in the left-hand portion of the pharmacophore to address issues of selectivity and oral bioavailability.[6c,d] This letter describes a series of benzo-fused heterocycles found to have greatly improved cellular potencies.

Keywords: Cathepsin S; Antigen processing; Enzyme inhibitor.
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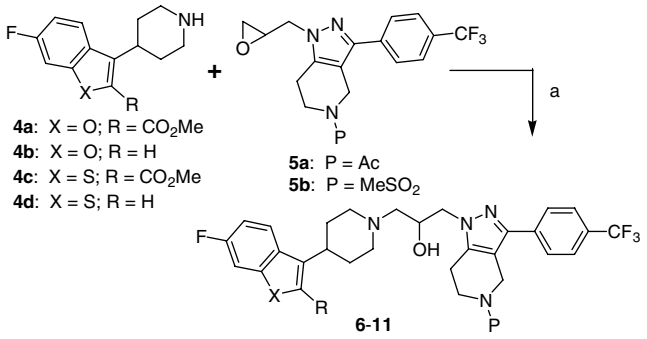
doi:10.1016/j.bmcl.2007.08.038

Following our previously described route, the known[9] benzofurans **4a**–**b** and benzothiophenes **4c**–**d** were prepared and coupled with epoxides **5a** and **5b** to afford the racemic target molecules **6**–**11** (Scheme 1). These molecules were moderately potent inhibitors of CatS (Table 1). Replacement of oxygen with sulfur had at best a minimal positive impact on CatS potency (**6** vs **7**; **8** vs **9**), while the carboxylate analogues showed a trend toward increased potency (**6** vs **8**; **7** vs **9**). Replacing the acetamide on the tetrahydropyridine ring with sulfonamide had little to no impact on potency (**7** vs **10**; **9** vs **11**). Although CatS activity was relatively insensitive to changes to the 'head group' heterocycle, the most potent analogue, compound **11**, provided a convenient handle to interrogate a previously unexplored region of the pharmacophore.

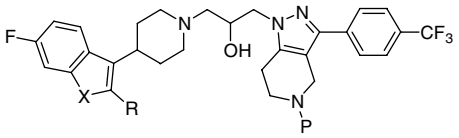
Initial attempts to transform the ester moiety of **11** to amide derivatives were thwarted by intramolecular capture of activated ester derivatives by the C2′ linker hydroxyl. We had previously observed[6c] that this hydroxyl substituent was unimportant for CatS potency, so we prepared the des-hydroxy analogue of **11**, compound **13** (Scheme 2). Thus, treatment of aldehyde **12** with piperidine **4c** in the presence of $NaBH(OAc)_3$ afforded the requisite ester **13** in good yield.

Functional group manipulation of the ester moiety of **13** afforded a series of amide analogues, and the CatS enzymatic potency and cellular data for these inhibitors were



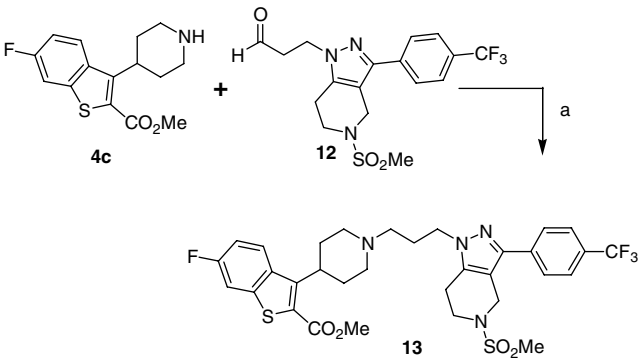
Scheme 1. Reagent and conditions: (a) EtOH, 60–70 °C (50–75%).

Table 1. Benzofuran/thiophene-based CatS inhibitors[a]



Compound	X	R	P	CatS IC_{50} (nM)
6	O	H	Ac	360
7	S	H	Ac	290
8	O	CO_2Me	Ac	220
9	S	CO_2Me	Ac	140
10	S	H	$MeSO_2$	180
11	S	CO_2Me	$MeSO_2$	130

[a] CatS IC_{50} and JY Ii degradation assay IC_{50} values are means of $n \geqslant 3$ runs and determined as described previously. All IC_{50}'s were within a 3-fold range.[6a] ND, not determined.

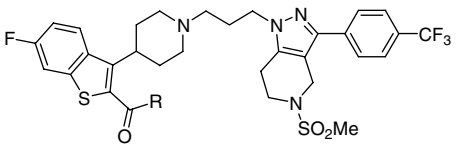


Scheme 2. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt (60%).

obtained. As shown in Table 2, changes to the C2 position of the benzothiophene impacted both enzymatic and cellular potency.

Although hydrolysis of the ester **13** to the acid **14** had no effect on CatS potency, cellular activity was abolished. Conversion of the methyl ester to the dimethyl amide (**15**) resulted in reduced CatS potency. Interestingly the ethyl amide **16**, isomeric with **15**, was 10-fold more potent (37 nM vs 370 nM), perhaps indicative of a favorable hydrogen bond interaction with the protein in this region of the pharmacophore. Compound **16** maintained good cellular activity (IC_{50} = 1.2 μM). Although encouraged by the activity of these new molecules, aqueous solubility was uniformly poor. The 2-aminoethyl analogue **17** and 2-(1-morpholino)ethyl analogue **18** were thus prepared. Although the solubility was little changed (data not shown), both **17** and **18** were potent CatS inhibitors. The addition of the amino moiety to **16** resulted in reduced cellular potency for **17**, while the morpholine analogue **18** was 3-fold more potent than **16** in the JY assay. Preliminary rat PK experiments on several analogues demonstrated low levels of oral bioavailability, however, and this series was not pursued further.

Table 2. Carboxylate/carboxamide CatS inhibitors[a,b]



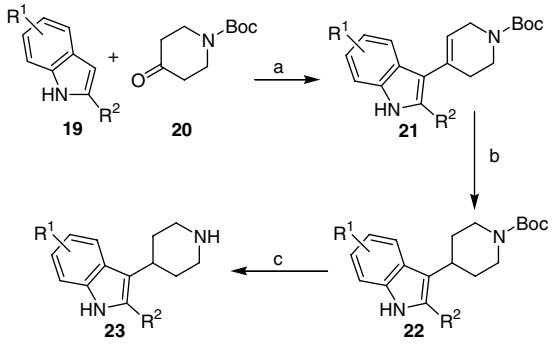
Compound	R	CatS IC_{50} (nM)	JY Ii IC_{50})μM)
13	OMe	130	1.8
14	OH	100	>10
15	NMe_2	370	ND
16	NHEt	37	1.2
17	$NHCH_2CH_2NH_2$	47	5.5
18	$NHCH_2CH_2R^*$	39	0.34

[a] CatS IC_{50} and JY Ii degradation assay IC_{50} values are means of $n \geqslant 3$ runs and determined as described previously. All IC_{50}'s were within a 3-fold range.[6a]

[b] R*, 1-morpholino.

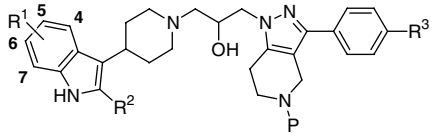
Whilst pursuing the benzofuran and benzothiophene analogues described above, we simultaneously prepared the analogous indole derivatives. Although the parent 4-(indol-3-yl)piperidine was commercially available, substituted analogues had to be synthesized (Scheme 3). Accordingly, condensation of an indole **19** with 1-*tert*-butoxycarbonyl-4-piperidone **20** afforded the tetrahydropyridines **21**. Reduction of the olefin by transfer hydrogenation and subsequent treatment with trifluoroacetic acid afforded the requisite indole-substituted piperidines **23** in excellent yields. Conversion to the target molecules proceeded as shown in Scheme 1.

The indol-3-yl piperidines demonstrated uniformly excellent CatS inhibition (Table 3). The parent analogue **24** had an IC_{50} of 72 nM that was only moderately affected by substitutions on the indole core. For example, the addition of a chlorine atom to C5′ (**25**) had no impact on CatS potency, while replacement of the chlorine atom in the sulfonamide analogue **27** with methoxy, methyl, cyano or ethyl carboxylate afforded potencies in a very tight range (21–58 nM; compounds **28**–**31**). The position of a chlorine atom on the indole nucleus had no impact on potency (**32** and **33**), nor did the addition of a methyl group at C2 of the indole (compound **34**). These observations are similar to what was observed in our earlier series of ketobenzimidazole derivatives.[6c]



Scheme 3. Reagents and conditions: (a) KOH, MeOH, reflux (85–95%); (b) Pd/C, NH_4HCO_2, MeOH, reflux (85–95%); (c) TFA, CH_2Cl_2, rt, (95+%).

Table 3. Effect of indole substitution on CatS activity[a]



Compound	R^1	R^2	R^3	P	CatS IC_{50} (nM)
24	H	H	CF_3	Ac	72
25	5-Cl	H	CF_3	Ac	95
26	5-Cl	H	Br	$MeSO_2$	54
27	5-Cl	H	CF_3	$MeSO_2$	44
28	5-MeO	H	CF_3	$MeSO_2$	29
29	5-Me	H	CF_3	$MeSO_2$	58
30	5-CN	H	CF_3	$MeSO_2$	21
31	5-CO_2Et	H	CF_3	$MeSO_2$	27
32	6-Cl	H	CF_3	$MeSO_2$	29
33	7-Cl	H	CF_3	$MeSO_2$	34
34	5-Cl	Me	CF_3	$MeSO_2$	37

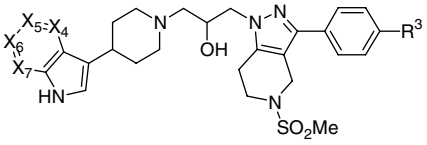
[a] CatS IC_{50} and JY Ii degradation assay IC_{50} values are means of $n \geqslant 3$ runs and determined as described previously. All IC_{50}'s were within a 3-fold range.[6a]

Although the indoles **24**–**34** all displayed excellent enzymatic potency, the cellular data were quite inconsistent. For example, compound **24** had an IC_{50} of 2.3 μM in our JY Ii assay, while the chloro analogue **25** was inactive at concentrations up to 10 μM, as were many of the other indoles (e.g., **28**, **29**, and **32**). Of the analogues tested, only the ethyl ester **31** had an IC_{50} below one micromolar in the JY Ii assay (IC_{50} = 410 nM).

The excellent enzymatic potency of the indoles combined with the high sensitivity of cellular activity to changes in functionality displayed in both the indole and benzothiophene series prompted us to consider further modifications of the indole core to improve cellular potency. Thus, we prepared azaindole analogues to gauge the impact of reduced lipophilicity of the indole moiety on activity. The requisite piperidines were prepared from all four azaindole isomers following the route depicted in Scheme 3, and the data obtained on the CatS inhibitors derived therefrom are tabulated in Table 4.

All four azaindoles were well tolerated by the enzyme, although the 4-aza analogue **35** was 2- to 3-fold less potent than the other isomers **36**–**38**. A much larger impact on cellular potency was observed. Although the weakest inhibitor of the enzyme, compound **35** had an IC_{50} of 95 nM in the cellular assay. The 5-aza analogue **36** and 6-aza analogue **37** were also quite potent at inhibiting p10 degradation in this assay, with IC_{50}'s of 25 nM and 38 nM, respectively. The anomaly in this set of molecules was the 7-aza derivative **38**. Although a very potent inhibitor of the enzyme (IC_{50} = 27 nM), compound **38** was only moderately active in JY cells (IC_{50} = 860 nM). The reasons for the decreased cellular potency of **38** relative to the isomers **35**–**37** are not readily

Table 4. Enzymatic and cellular potency of azaindole analogues[a]



Compound	X_4	X_5	X_6	X_7	R^3	CatS IC_{50} (nM)	JY Ii IC_{50} (μM)
35	N	H	H	H	CF_3	120	0.095
36	H	N	H	H	CF_3	58	0.025
37	H	H	N	H	CF_3	30	0.038
38	H	H	H	N	CF_3	27	0.86
39	H	N	H	H	Br	77	0.15
40	H	H	H	N	Br	195	ND
41	H	N^+–O^-	H	H	CF_3	200	0.24

[a] CatS IC_{50} and JY Ii degradation assay IC_{50} values are means of $n \geqslant 3$ runs and determined as described previously. All IC_{50}'s were within a 3-fold range.[6a]

apparent. Screening of other related proteases (CatB, CatE, CatL, legumain) showed no inhibition at concentrations up to 5 μM, for those analogues tested.

Anticipating that the pyridine nitrogen of the azaindole would be a metabolic liability, the pyridine *N*-oxide of **36**, compound **41**, was also prepared. This molecule was significantly less active than **36** in both the enzymatic and cellular assays, although the cellular potency of 240 nM was very competitive with previously reported analogues.[6]

After this work was completed, a report from Merck-Frosst demonstrated that certain cathepsin K (CatK) inhibitors with moderate activity against CatS enzyme displayed significant levels of CatS inhibitory activity in cellular assays.[10] In an elegant series of experiments, these investigators demonstrated that covalent, reversible CatK inhibitors with basic moieties (e.g., piperazines) accumulated in lysosomes both in vitro and in vivo (rat whole-body radiography), thus leading to a loss in cellular selectivity. Similar experiments have not been performed on the molecules reported in this letter, although the improved cellular potency of the azaindoles (Table 4) compared to the indoles (Table 3) may be indicative of lysosomotropism.

In conclusion, bioisosteric replacement of the headgroup ketobenzimidazole piperidines with 4-(indol-3-yl) piperidines resulted in a new series of CatS inhibitors with greatly improved cellular potency. Determining the reason for the disparity between enzymatic potency and cellular potency for these noncovalent CatS inhibitors still requires further investigation. The utility of these novel CatS inhibitors in elucidating the pharmacology of CatS, and its potential as a target for immunosuppressive therapies, will be reported in due course.

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